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13. ABSTRACT (Maximum 200 Words)

Plasmin enzymatically produces an Osteocalcin-derived C-terminal Hexapeptide from bone matrix which promotes the growth of human osteosarcoma cells by blocking the action of the hormone oxytocin.

We hypothesize that the C-terminal Hexapeptide promotes the growth of breast cancer cells in bone. Breast cancer cells that are growth inhibited by oxytocin may be growth stimulated in bone where abundant C-terminal Hexapeptide is present. Growth stimulation in bone would enhance the likelihood of bone metastasis by breast cancer cells.

Our first objective was to determine the affect of C-terminal Hexapeptide on the growth of MDA-MB-231 breast cancer cells inhibited by oxytocin, as published by others. Oxytocin (10^{-5} M)inhibited growth but this is not in agreement with published results. We have obtained a 1-year no cost extension to: examine alternate sources and forms of oxytocin and try cAMP and PGE_2 assays to see if biological effectiveness of oxytocin is achieved. Once an effective oxytocin and cell assay is established we will characterize the effects of hexapeptide on breast cancer cells.

Counteracting the metastasis of breast cancer to bone could enhance patient survival. Understanding the mechanisms that enable breast cancer cells to metastasize, survive, and grow in bone are vital to alter the process. The experiments should provide new information on the growth stimulatory environment of bone, and contribute to the understanding of bone-seeking breast cancers. The knowledge will be useful for developing strategies to counteract the spread of breast cancer to bone.

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Introduction

This laboratory has shown that the protease Plasmin enzymatically produces an Osteocalcin-derived C-terminal Hexapeptide from bone matrix [1]. This hexapeptide promotes the growth of human osteosarcoma cells by blocking the action of the hormone oxytocin [2].

We hypothesize that by the same mechanism C-terminal Hexapeptide promotes the growth of breast cancer cells in bone.

Rationale: Osteocalcin, an abundant protein of the bone matrix, is metabolized to form two peptides, the carboxy-terminal hexapeptide (RFYGPV) called "C-terminal Hexapeptide" and an amino terminal "N-mid peptide" [1]. We have proven that the C-terminal Hexapeptide and N-mid peptide are formed by Plasmin proteolysis [1]. Plasmin is postulated to be involved in tumor metastasis due to the presence of plasminogen activators on the tumor cell surface [2]. The N-mid peptide is the most abundant metabolite of human Osteocalcin [3]. In the same metabolic cleavage, the C-terminal Hexapeptide is constantly being made as a normal metabolite of Osteocalcin. We have proven that the C-terminal Hexapeptide is growth stimulatory for osteosarcoma cells, perhaps mediating the growth of osteosarcoma within the bone [2]. The C-terminal Hexapeptide blocks oxytocin action [2]. Human breast cancer cells that are growth inhibited by oxytocin [4,5] may be growth stimulated in bone where abundant C-terminal Hexapeptide is present. Growth stimulation in bone would enhance the likelihood of bone metastasis by breast cancer cells.

Counteracting the metastasis of breast cancer to bone could enhance patient survival. Understanding the mechanisms that enable breast cancer cells to metastasize, survive, and grow in bone are vital to alter the process. It may be possible to develop strategies to inhibit C-terminal Hexapeptide growth enhancement to block metastasis of breast cancer to bone.

Body

The initial objective was to determine the affect of C-terminal Hexapeptide (available as synthetic peptide) on breast cancer cell growth that was inhibited by oxytocin. Initial experiments were to establish culture conditions to reproduce previous results [4,5]. Cultured human breast carcinoma cells (MDA-MB-231 from American Type Culture Collection) were tested for growth effects by adding oxytocin and assessing the effect on growth by the ATCC MTT colorimetric assay. The effect of cell number and serum concentration on the growth inhibitory effect of oxytocin were determined. 1×10^{-5} M oxytocin inhibited growth of MDA-MB-231 only in the presence of 2% serum not 10% serum. Previously, 1×10^{-7} M has been published as an effective dose [4,5]. We have tried different batches and forms of oxytocin to determine whether there is a more active form for our studies. These efforts are ongoing. We are establishing an assay for cAMP and/or PGE_2
as well as growth to assess the effectiveness of oxytocin on the cells. We have been granted a one-year no cost extension to continue our studies.

Key Research Accomplishments

[1] After a short delay a half-time technician has been trained for culture experiments and growth assays. The laboratory was not previously culturing cells or performing growth assays, but is now competent in both techniques.

[2] The activity of oxytocin preparations has been discovered to vary with type and purity. The laboratory is still searching for a reliable source of oxytocin, and is developing methods to assess activity prior to committing to time-consuming growth curve experiments.

Reportable Outcomes

Based on the work from this grant, the NIH grants "Oxytocin promotes bone metastases by breast carcinoma cells", (submitted for 6/1/02) and "Oxytocin promotes bone metastases" (submitted for 11/21/02) were submitted. A DOD Exploration Award proposal "Promotion of breast cancer metastases to bone by oxytocin receptor and ostercalcin" (submitted for 6/13/02) was submitted.

Conclusions

Our experiments have been inconclusive because the variable activity of oxytocin in our assay. The cell growth assay is working well with very good reproducibility. Oxytocin growth inhibition of MDA-MB-231 breast cancer cells has been difficult to achieve with reproducibility. In some experiments a growth stimulation has been observed. Although this is interesting in light of other cell lines that are growth stimulated by oxytocin, the difficulty in achieving reproducible effects has thwarted attempts to test the activity of C terminal Osteocalcin peptide. During the extension period, we have explored another member of the osteocalcin-family member named matrix gla protein and its role in cell adhesion to extracellular matrix. Cell adhesion is relevent to the process of cancer metastasis, and will be a future research area for the laboratory.

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Appendix

none